

AMENDMENTS

In the Claims:

1. (Previously presented) A method for detecting and counting the microorganisms in a sample comprising the steps of:
 - a) selectively enriching the microorganism sought in the sample,
 - b) inducing or activating at least one enzymatic activity of the aforementioned microorganism,
 - c) immunomagnetically concentrating the enzymatically activated microorganism,
 - d) fluorescently labeling the concentrated microorganism obtained after immunomagnetic concentration is carried out by adding to the medium containing the aforementioned microorganism at least one substrate comprising one part specific to the enzymatic activity to be indicated and one label part, the transformation of the substrate takes place inside the aforementioned microorganism and that the fluorescent product is retained in the aforementioned microorganism, and
 - e) detecting and analyzing the fluorescence making possible the numeration or counting of the microorganisms sought is carried out by a technique chosen from the group comprising: flow cytometry, filtration cytometry and fluorescence microscopy.
2. (previously presented) A method according to claim 1, wherein the enrichment step is carried out in a composition comprising:

sodium pyruvate at a concentration selected from the group consisting of between 1 and 20 g/L, between 1 and 10 g/L, and between 4 to 6 g/L,

sodium thiosulfate at a concentration selected from the group consisting of between 0.5 and 5 g/L, between 0.5 and 3 g/L, and approximately 2 g/L,

catalase at a concentration selected from the group consisting of between 500 and 20,000 μ /L, between 2,000 and 8,000 μ /L, and approximately 5,000 μ /L.

3. (previously presented) A method according to claim 2, wherein said composition comprises in addition at least one antibiotic.
4. (previously presented) A method according to claim 1, wherein the conditioning step is an induction step for at least one enzymatic activity specific to the microorganism sought, comprising adding to the microorganism's enrichment medium at least one non-fluorescent substrate specific to the aforementioned enzyme or enzymes.
5. (original) A method according to claim 4, wherein steps a) and b) can be carried out simultaneously.
6. (original) A method according to claim 4 or 5, wherein step c) can take place before step b) or step c) can take place after step d).
7. (original) A method according to claim 1, wherein the conditioning step, in the case where the microorganism sought is a Gram-positive bacteria, comprises in addition an induction step for at least one surface antigen characteristic of the microorganism sought, comprising adding to the microorganism's enrichment, medium yeast extract at a concentration selected from the group consisting of between 5 and 50 g/L, between 10 and 20 g/L, and approximately 10 g/L.
8. (previously presented) A method according to claim 1, wherein the immunomagnetic concentration step comprises the steps of:

- a) placing the microorganism sought, present in the conditioning medium, in contact with an antibody directed against an antigen specific to the microorganism, the aforementioned antibody being conjugated with a magnetic bead,
- b) separating the bead-antibody-microorganism complexes from the medium, and
- c) separating the microorganism from the rest of the complex.

9. (original) A method according to claim 8, wherein the antibody conjugated with a magnetic bead is directed against an antibody that is itself directed against an antigen specific to the microorganism sought.

10. (previously presented) A method according to claim 8 or 9, wherein the magnetic beads have a diameter that is between 1 and 20 μm , or between 2 and 8 μm .

11. (previously presented) A method according to claim 1, wherein fluorescent labeling of the microorganisms sought is carried out by adding to the medium containing said microorganisms at least one substrate comprising a part specific to the enzymatic activity to be revealed and one label part.

12. (previously presented) A method according to claim 11, wherein the label is a fluorogenic label excited at 488 nm selected from the group consisting of the xanthenes, acridines, phycobiliproteins, cyanine, and esculin.

13. (previously presented) A method according to claim 11, wherein the substrate part specific to the enzymatic activity to be revealed is selected from the group consisting of a fatty acid, a monosaccharide, a phosphate, and a sulfate.

14. (previously presented) A method according to claim 1, wherein the detection and analysis of fluorescence are carried out by a technique selected from the group consisting of flow cytometry, filtration cytometry and fluorescence microscopy.

15. (previously presented) A method according to claim 1, wherein steps a), b), c), d), and e) are preceded by a filtration step for the sample to be analyzed.

16. (previously presented) A method according to claim 15, wherein the filtration is carried out by means of a filter whose porosity is a size selected from the group consisting of between 20 and 150 microns, between 30 and 100 microns, and approximately 63 microns.

17. (previously presented) A method according to claim 15, wherein the filtration is carried out on a membrane presenting a porosity selected from the group consisting of between 0.2 and 10 μm , preferably between 0.2 and 5 μm , and between 0.2 and 0.5 μm .

18. (withdrawn) A selective enrichment medium for a microorganism sought in a sample comprising:

a nutrient composition making the multiplication of said organism possible,

and

a selective revivification composition for said microorganism, wherein it comprises:

sodium pyruvate at a concentration selected from the group consisting of between 1 and 20 g/L, between 1 and 10 g/L, and between 4 to 6 g/L,

sodium thiosulfate at a concentration selected from the group consisting of between 0.5 and 5 g/L, between 0.5 and 3 g/L, and approximately 2 g/L,

catalase at a concentration selected from the group consisting of between 500 and 20,000 μL , between 2,000 and 8,000 g/L, and approximately 5,000 μL .

19. (withdrawn) An enrichment medium according to claim 18, which further comprises at least one antimicrobial agent.

20. (withdrawn) A kit for detecting and counting microorganisms comprising:

a) an enrichment medium according to claim 18 in a liquid or dehydrated form, a plastic bag lined with a full-surface filter presenting a porosity of approximately 63 μm ,

b) magnetic beads conjugated to an antibody specific for an antigen on said microorganism,

c) one or several substrates in a lyophilized form,

d) solvents,

wherein said substrates of part (c) comprises a part specific to enzymatic activity to be revealed and a label.